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Review Paper

Participation of group I p21-activated kinases in neuroplasticity

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ABSTRACT

PAKs are a family of serine/threonine protein kinases activated by small GTPases of the Rho family, including Rac and Cdc42, and are categorized into group I (isoforms 1, 2 and 3) and group II (isoforms 4, 5 and 6). PAK1 and PAK3 are critically involved in biological mechanisms associated with neurodevelopment, neuroplasticity and maturation of the nervous system, and changes in their activity have been detected in pathological disorders, such as Alzheimer's disease, Huntington's disease and mental retardation. The group I PAKs have been associated with neurological processes due to their involvement in intracellular mechanisms that result in molecular and cellular morphological alterations that promote cytoskeletal outgrowth, increasing the efficiency of synaptic transmission. Their substrates in these processes include other intracellular signaling molecules, such as Raf, Mek and LIMK, as well as other components of the cytoskeleton, such as MLC and FLNa. In this review, we describe the characteristics of group I PAKs, such as their molecular structure, mechanisms of activation and importance in the neurobiological processes involved in synaptic plasticity.

Contents

1. Introduction: Kinase activated by p21 (PAK)

PAKs are proteins involved in several intracellular processes, such as signal transduction, cellular motility and cytoskeletal protein synthesis (Bokoch, 2003; Kumar et al., 2006). These proteins were first identified by Manser 1994 as effectors of the Rho family of small GTPases, specifically cell division cycle 42 (Cdc42) and

Abbreviations: AID, self-inhibitory domain; Cdc42, cell division cycle 42; CREB, cyclic adenosine monophosphate response element-binding protein; CRIB, Cdc42/Rac interactive binding; Erk, extracellular signal-regulated kinase; FLNa, filamin A; LIMK, LIM kinase; MLCK, myosin light chain kinase; PAK, kinase activated by p21; PBD, p21-binding domain.

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Ras-related C3 botulinum toxin substrate (Rac). The Rho family of small GTPases becomes inactivated when bound to guanosine diphosphate (GDP) and activated when bound to guanosine triphosphate (GTP). In the latter form, Rho GTPases drive several intracellular molecular activities and directly bind to PAKs, allosterically modifying their structure and facilitating their phosphorylation via a third protein (Ong et al., 2002; Shin et al., 2013; Wang et al., 2013).

PAKs are a serine/threonine kinase protein family consisting of two relatively distinct subfamilies: group I, consisting of isoforms 1, 2 and 3, and group 2, consisting of isoforms 4, 5 and 6. The expression of PAK isoforms from both groups have been detected in the mammalian brain and other tissues, but aside from their structural and genetic similarities, these groups of PAKs are distinct and have different functions (Manser et al., 1995; Hofmann et al., 2004; Shin et al., 2013). Group I PAKs display high amino acid sequence similarity, and many pieces of evidence gathered in recent years suggest that this group plays unique roles in brain ontogenesis, neuronal differentiation and synaptic plasticity (Kreis et al., 2007).

Small GTPases promote allosteric regulation of PAKs in a manner that facilitates their phosphorylation and, therefore, their activation (Edwards et al., 1999; King et al., 2001). Once this occurs, PAKs perform their kinase activity on other serine/threonine kinases, such as the proto-oncogene c-Raf (Raf), extracellular signal-regulated kinase (ERK) kinases (MEKs), LIM kinase (LIMK) and myosin light chain kinase (MLCK), positively regulating actin protein synthesis and polymerization, essential events in the process of neuroplasticity (Goeckeler et al., 2000; King et al., 2001; Somlyo and Somlyo, 2003; Zang et al., 2008).

Several studies have revealed evidence linking the PAKs to pathological disorders, such as cancer, Alzheimer's disease, mental retardation and Huntington's disease (Luo et al., 2008; Ma et al., 2008; Minden, 2012; Ye and Field, 2012). PAK is also implicated in type 2 diabetes, since islets from type 2 diabetic individuals contain 80% less PAK1 protein than non-diabetic individuals (Kalwat et al., 2013). Moreover, a recent study demonstrated that this kinase plays an important role in gene expression modulations that were associated with DNA damage induced by ionizing radiation (Motwani et al., 2013).

In this review, we aim to provide information about the group I PAKs, isoforms 1, 2 and 3, concerning the following topics: their molecular structure, activation, localization in the neural system, substrates and importance in neural plasticity and learning.

2. Molecular structure

The group I PAKs include tree isoforms: PAK1, 2 and 3. These isoforms display 93% similarity in the amino acid sequence (Bokoch, 2003). Isoforms 1, 2 and 3 have a molecular weight of 68, 62 and 65 kDa, respectively. PAKs from both groups (I and II) contain an N-terminal regulatory domain and a C-terminal catalytic domain, which are the key sites for the regulation of their kinase activity by Rho GTPases and other kinases that phosphorylate PAKs (Manser et al., 1994; Bokoch, 2003).

The N-terminal region of PAKs contain a p21-binding domain (PBD), which possesses the binding site of the Rho family of small GTPases, including Cdc42 and Rac, termed the Cdc42/Rac interactive binding (CRIB) motif, as well as several polyproline (PXXP) motifs, which serve as binding sites of proteins that possess a Src homology 3 (SH3) domain, such as the Nck adaptor proteins, interacting exchange factor (bPIX) and growth factor receptor-bound protein 2 (Grb2) (Coniglio et al., 2008). The SH3 domain is commonly found in proteins that interact with other proteins to mediate the assembly of specific signaling complexes typically by binding to proline-rich regions of a binding partner (Coniglio et al., 2008; Deacon et al., 2008; Coleman and Kissil, 2012).

In the PAK C-terminal (catalytic) region, there is a highly conserved kinase domain that allows PAK to phosphorylate its substrates (Manser et al., 1998; Hofmann et al., 2004; Wang et al., 2011). Furthermore, in the N-terminal (regulatory) region of only group I PAKs, a self-inhibitory domain (AID), which is the source of the self-inhibitory capacity of PAK. In the inactive form, the AID of PAK binds to and downregulates the activity of the catalytic domain of other PAK molecules, acting as a trigger that regulates the basal activity of the kinase (Chong et al., 2001; Arias-Romero and Chernoff, 2008; Wang et al., 2011).

When inactive, the group I PAKs are organized as homodimers; in other words, two identical PAK isoforms are fused. This homodimer remains stable, with the N-terminal region of one PAK molecule overlying the C-terminal region of the other one (Bokoch, 2003). Under this condition, the PBD of each molecule comprising the dimer is partially twisted behind its AID, preventing the phosphorylation of Thr423 in the activation loop by other kinases (Chong et al., 2001; Arias-Romero and Chernoff, 2008; Wang et al., 2011). PAK must undergo an allosteric conformational modification to expose the Thr423 motif, which allows for its phosphorylation by other kinases to activate the kinase. Therefore, the important role of these signaling pathways is clear.

3. Mechanism of action

PAKs are kinases that serve as important effectors of GTPases, especially Cdc42 and Rac (Boda et al., 2006; Arias-Romero and Chernoff, 2008; Chi et al., 2013). The Rho family of GTPases participates in intracellular signaling pathways as a molecular trigger with many downstream targets. Among them, PAKs (Ong et al., 2002) are importantly associated with cellular morphological alterations, such as protein synthesis processes, actin polymerization and lamellipodia and filopodia projections (Etienne-Manneville and Hall, 2002; Sinha and Yang, 2008).

GTPases become activated when bound to GTP and inactivated when bound to GDP. GTPases are crucial for several cellular processes, such as cellular differentiation and proliferation, cytoskeletal organization, vesicle trafficking and nuclear transport (Etienne-Manneville, 2004). The mechanism of action of GTPases begins when a guanine exchange factor (GEF) disrupts the molecular interaction between the GTPase and GDP, facilitating the interaction between GTP and the GTPase. The opposite occurs when a GTPase-activating protein (GAP) promotes the hydrolysis of GTP to form GDP, inactivating the Rho GTPase (Etienne-Manneville and Hall, 2002; Bokoch and Zhao, 2006; Sinha and Yang, 2008; Chi et al., 2013).

When bound to GTP, Cdc42 and Rac associate with homodimer PAK molecules in the N-terminal region of the CRIB domain, causing a conformational alteration and destabilizing the dimerization, leading to the decoupling of the monomers (Galisteo et al., 1996; Bokoch, 2003; Li et al., 2003; Coleman and Kissil, 2012). This destabilization of the homodimer relies on the release of the AID via the binding of a Rho GTPase to the PBD of a molecule comprising the homodimer, which then dissociates from the catalytic region of the other molecule. Both molecules undergo this allosteric modification induced by Cdc42 or Rac to mutually release themselves (Coleman and Kissil, 2012; Shin et al., 2013).

Several PAK phosphorylation sites, such as Ser149, Ser198, Ser203 and Thr423 are associated with AID release (Zenke et al., 1999). This modification occurs due to the conformational change in the dimer caused by its interaction with Cdc42 and Rac. Thr423 has been considered as the most critical site that inhibits AID binding and stabilizes the molecule in its active phosphorylated form. The exposure of the Thr423 site in the activation loop provides a target for other kinase molecules via the key effector function of

phosphoinositide-dependent kinase-1 (PDK1) (Zenke et al., 1999; Lei et al., 2000; Coleman and Kissil, 2012; Eswaran et al., 2012).

PAK1 is also stimulated by phosphorylation at Ser21 and Thr212. Akt phosphorylates PAK1 at Ser21 independently of the binding of Rho GTPases; this phosphorylation induces the release of the Nck adapter protein from the N-terminal region of PAK1 and its translocation to the cytosol (Thiel et al., 2002; Zhou et al., 2003). Residue Thr212 of PAK1 is known to be a common target of cyclin-dependent protein kinase (Cdc2) and cyclin-dependent kinase 5 (Cdk5). Phosphorylation by Cdc2 at Thr212 is detected in mitotic cells and is critically associated with the morphological changes involved in cell division (Thiel et al., 2002), whereas phosphorylation of the same residue by Cdk5 in neurons appears to regulate the activity of PAK, a key event in the cytoskeletal remodeling of growing axons (Rashid et al., 2001).

4. Localization in the nervous system of mammals

Previous studies have demonstrated that group I PAKs are differentially distributed among tissues. Whereas PAK1 is present in the brain and the spleen, PAK2 is equally distributed throughout several tissues and PAK3 is predominantly expressed in the brain (Manser et al., 1995; Teo et al., 1995; Rousseau et al., 2003).

In the brain, PAK1 is found to be most strongly expressed in the hippocampal region, primarily in the CA1 region, the cortex (primarily in layers IV and V), the cerebellum, the medulla, the piriform cortex and the ventral/lateral thalamic nuclei. PAK3 is also expressed in these regions, but it is most strongly expressed in the hippocampal dentate gyrus and layers II, III and V of the cortex, as well as the olfactory bulb, the piriform cortex, the medial preoptic nucleus, the hypothalamus, the thalamus, the amygdala and the raphe nuclei (Manser et al., 1995; Allen et al., 1998; McPhie et al., 2003; Boda et al., 2006). PAK3 was detected in cells during their division cycle in the cerebral ventricles and in the subventricular zone (Teo et al., 1995). Previous studies have demonstrated that PAK isoforms 1 and 2 are more strongly expressed in oligodendrocytes, whereas PAK3 is more strongly expressed in neurons, and all three isoforms are strongly detected in the brain (Arias-Romero and Chernoff, 2008; Cahoy et al., 2008; Kreis et al., 2008).

The group I PAK isoforms are also differentially localized within neurons. The presence of this kinase has been detected in dendritic spines, and its active form is highly elevated in the postsynaptic density (Hayashi et al., 2002, 2004). In hippocampal and cortical neurons, PAK1 is highly concentrated in axons and dendrites, whereas PAK3 is more highly concentrated in cell bodies and dendrites of larger diameter (Hayashi et al., 2002; Ong et al., 2002). In a study performed using cultured N1E – 115 neuroblastoma cells, PAK1 expression predominated along the neurite axes and the cell center, PAK2 expression predominated in filopodia and filopodial activity areas, and PAK3 expression was found in areas containing lamellipodia and membrane ruffling, such as the newly polymerized actin mesh (Marler et al., 2005).

The expression levels of these kinases are influenced by various factors under both normal and pathological conditions (Kumar et al., 2006). The expression of PAK1 is elevated in primary cortical neurons derived from mouse embryos, and its expression level is maintained until adulthood; alternatively, the expression of the PAK 3 is upregulated during neuronal differentiation and migration of GABAergic interneurons in the rat cortex (Souopgui et al., 2002; Cobos et al., 2007; Causeret et al., 2009).

Analysis of brain tissue from post-stroke survivors 2 or 6 days after ischemia revealed upregulation of PAK1 (Mitsios et al., 2007). The same result was detected in tissues obtained within 4 weeks after the ischemic event. PAK1 was also upregulated in rat brain tissues obtained 1, 12 or 24 h after ischemia, but the expression level of this kinase returned to baseline 7 days after the ischemic event (Mitsios et al., 2007). In another study, it was found that in genetically modified rats more prone to depression, PAK1 was downregulated in the frontal cortex compared animals resistant to depression (Nakatani et al., 2007).

5. Target substrates

PAK is associated with several intracellular mechanisms that are involved in many biological processes. The participation of PAK in those processes occurs via many molecules that act upstream and downstream of it. PAK becomes activated by the allosteric regulatory mechanism described above or by phosphorylation in the activation loop, which leads to the activation of other signaling molecules via the kinase activity of PAK (Bokoch, 2003; Boda et al., 2006; Eswaran et al., 2012).

The Erk pathway is an important pathway that is associated with PAK. Rho GTPases upregulate the Erk pathway, which consists of three kinases which are arranged from the cell membrane toward the nucleus in the following order: Raf, Mek and Erk, such that Raf phosphorylates Mek, and Mek phosphorylates Erk. The Erk pathway is a modulator of the cytoskeletal morphological changes in several tissues, including nervous tissue (King et al., 1998; Zang et al., 2002; Yi et al., 2010). This pathway is activated by Rho GTPases bound to GTP, leading to an increase in Erk activity via cyclic adenosine monophosphate response element binding protein (CREB). When phosphorylated, CREB induces the transcription of genes important for neuronal plasticity processes (Frost et al., 1997; Park et al., 2007; Cao et al., 2012).

Many studies have demonstrated that Mek1 and Raf1 are two direct substrates of PAK. Raf1 becomes activated upon phosphorylation at Ser 338, and phosphorylation of Mek1 at Ser298 facilitates signal transduction from Raf to Mek. Thus, Mek1 becomes activated (King et al., 1998; Zang et al., 2002; Yi et al., 2010; Field and Manser, 2012). Co-expression of constitutively active PAK and Raf can replace Rho GTPases as an activator of Erk, thus increasing the phosphorylation levels of this molecule (Frost et al., 1997). PAK also directly phosphorylates Mek at Ser298 in vitro and *in vivo* and elevates the level of human Mek phosphorylation at Thr292; both of these sites are essential for the Raf-Mek interaction (Frost et al., 1997; Park et al., 2007; Yi et al., 2010; Wang et al., 2013).

It was also demonstrated that inhibition of group 1 PAKs reduces Raf phosphorylation at Ser338 and Mek phosphorylation at Ser298 via platelet-derived growth factor (PDGF) and epithelial growth factor (EGF) (Manser and Zhao, 2012). PAK is also associated with the activity of LIMK, a serine/threonine kinase that promotes actin polymerization via cofilin inhibition (Edwards et al., 1999). While dephosphorylated, cofilin is active and downregulates actin polymerization. This condition is reversed by LIMK, which phosphorylates cofilin, inactivating it and preventing actin depolymerization (Moon and Drubin, 1995; Lamprecht and LeDoux, 2004; Rubio et al., 2012; Deo et al., 2013).

Prior studies have found that PAK can activate LIMK via Thr508 phosphorylation, increasing the capacity of LIMK to phosphorylate cofilin at Ser3 (Edwards et al., 1999). Therefore, the Rac and Cdc42 GTPases are associated with the actin cytoskeletal structure by regulating its polymerization level via the kinase activity of PAK and LIMK on cofilin (Bokoch, 2003; Rubio et al., 2012; Aslan and McCarty, 2013; Deo et al., 2013).

Another group of mechanisms in which PAKs appears to be involved is the dynamic regulation of actin (Boda et al., 2006). Several studies have demonstrated that PAK plays a key role in myosin light chain (MLC) activity modulation in hippocampal dendritic spines as well as in mammal fibroblasts (Wirth et al., 2003;

Zhang et al., 2005). MLC is found in dendritic spines and converts the energy from ATP hydrolysis to actin–myosin kinetic force. PAK and MLC kinase (MLCK) phosphorylate MLC at Ser19, activating myosin ATPase (Goeckeler et al., 2000; Wirth et al., 2003; Deo et al., 2013). MLC phosphorylation is a critical factor in the long-term structural stability of the actin cytoskeleton and underlies molecular processes that lead to long-term memory consolidation (Zhang et al., 2005; Rubio et al., 2012). Such findings suggest the importance of PAKs in synaptic morphogenesis and cellular motility (Sells et al., 1999).

On the other hand, PAK also indirectly inhibits MLC activity via direct MLCK phosphorylation. When MLCK is phosphorylated by PAK at Ser 439 and 991, its upregulatory effect on MLC is decreased, thereby reducing stress fiber formation and limiting isometric tension development in smooth muscle and non-muscle cells (Goeckeler et al., 2000; Coniglio et al., 2008; Szczepanowska, 2009). However, the mechanism by which PAK regulates these pathways in a temporal and spatial manner during cell motility is not well understood (Coniglio et al., 2008; Szczepanowska, 2009).

PAK1 has well known importance in stabilizing actin in the cellular membrane via the phosphorylation of filamin A (FLNa) at Ser2152. FLNa binds to actin and organizes it into orthogonal filaments attached to the cell membrane in motile cells. Because it appears that FLNa may be anchored to the CRIB domain of PAK1, stimulating its kinase activity, it can be concluded that this interaction contributes to the local activation of PAK1 (Vadlamudi et al., 2002; Szczepanowska, 2009; MacPherson and Fagerholm, 2010).

6. Cytoskeletal regulation

Several studies have provided evidence for the important roles of PAK in cytoskeletal dynamics due to its activity in pathways related to actin polymerization, novel protein synthesis and intracellular movement (Arias-Romero and Chernoff, 2008). The earliest evidence that PAK could be involved in the regulation of the cytoskeletal dynamics arose in 1997, when Sell induced PAK overexpression in Swiss 3T3 quiescent cells and detected an increase in lamellipodia and filopodia formation. Furthermore, the expression of PAK in its constitutively active form leads to a reduction in actin stress fibers and an increase in cellular motility (Bloom et al., 2003; Smith et al., 2008; Szczepanowska, 2009).

When PAK is activated by Rac/Cdc42, it induces the formation or remodeling of lamellipodia, filopodia, membrane ruffles, stress fibers and focal adhesion complexes (Bokoch, 2003). The interaction between PAK and Rac/Cdc42 triggers mechanisms that lead to alterations in the properties of proteins involved in cytoskeletal restructuring, including actin/myosin intermediary filaments, microtubules, integrins among other proteins (Brzeska et al., 1997; Kelly and Chernoff, 2011; Chi et al., 2013).

Actin is an essential cellular component to the maintenance of cellular dynamics. Thus, it is crucial for many processes ranging from motility, cell division and morphogenesis, as well as intracellular protein trafficking. The actin cytoskeleton plays a key role in neuronal development and synaptogenesis. Additionally, actin is the most predominant component of the presynaptic and postsynaptic cytoskeleton of mature neurons (Landis et al., 1998; Hirokawa et al., 1989; Bloom et al., 2003). In addition, actin is expressed at high levels in dendritic spines, the postsynaptic structure specialized for excitatory synaptic transmission (Matus, 2000; Capani et al., 2001; Yuste and Bonhoeffer, 2004).

The participation of PAK in the mechanism of cytoskeletal rearrangement has been typically considered as a result of indirect kinase activity on cell structural components. PAK phosphorylates LIMK at Thr508, increasing its phosphorylation and cofilin inactivation (Bokoch, 2003; Cingolani and Goda, 2008; Kelly and Chernoff, 2011). LIMK phosphorylates cofilin at Ser3, inactivating and preventing its coupling to the F-actin bearded end, which facilitates actin polymerization (Manser et al., 1995; Cingolani and Goda, 2008; Szczepanowska, 2009; Sit and Manser, 2011), strongly highlighting the importance of PAK in the pathway of actin regulation in neurons.

There are many PAK substrates that participate in its modulatory effect on cytoskeleton rearrangement. Some of these substrates are proteins that comprise the cytoskeleton. Among these cytoskeletal components which are targets of PAK are the myosins, which belong to a protein superfamily that shares the same actin–interacting domain, hydrolyze ATP and generate movement (Sellers, 2000; Redowicz, 2007).

The MLC is an essential component of this family that is responsible for cytoskeletal dynamics for communication, migration and cell division (Sellers, 2000; Redowicz, 2007; Hartman et al., 2011) Myosin has an important role in the processes related to structural stabilization and cellular dynamics, rendering the cell membrane resistant to potential deformations. Furthermore, myosin is involved in actin-enabled motor mechanisms and the organization of actin in the intracellular space (Hartman et al., 2011; Kneussel and Wagner, 2013).

FLNa, a substrate of PAK, is also an important cellular structural component. FLNa associates with actin and organizes its filaments as orthogonal networks bound to the cellular membrane, providing the necessary actin stabilization during cellular motor activities. FLNa is phosphorylated by PAK1 at the Ser2152 residue, a process that is necessary to attain its full activation and its efficacy to interact with actin. The benefits of the interaction between PAK1 and FLNa are mutual, as FLNa also anchors to the CRIB domain of PAK and stimulates its kinase activity, thus highlighting the importance of this interaction to the local activation of PAK (Goeckeler et al., 2000; Szczepanowska, 2009; MacPherson and Fagerholm, 2010).

In mammalian neurons, PAK1 and 3 phosphorylate MLC at Ser19, promoting dendritic spine morphogenesis via local actin network stabilization (Ramos et al., 1997; Chew et al., 1998; Van Eyk et al., 1998; Kneussel and Wagner, 2013). Accordingly, previous studies using cultured hippocampal cells have demonstrated that constitutively activated PAK1 and 3 lead to an increase post synaptic density (PSD) formation. The number of dendritic spines in neurons constitutively active expressing PAK1 was increased by 39% compared to control neurons, whereas that of dendritic protrusions were increased by 176%. Furthermore, there was a concomitant increase in the number of PSD protein clusters, suggesting an increase in the formation of excitatory synapses (Zhang et al., 2005).

Nevertheless, the expression of PAK isoforms 1 and 3 containing a mutation in their kinase domain caused a significant decrease in the number of dendritic spines and PSDs (Zhang et al., 2005). These mutants most likely do not efficiently bind to GTPases, such as Rac or Cdc42, and in addition, their activation and interaction with other effectors involved in synaptic plasticity is impaired (Sells et al., 1997; Tang et al., 1997).

Cytoskeletal rearrangement processes, as well as the outgrowth of cellular machinery, such as increased expression of signaling proteins and intracellular trafficking to their sites of activity, depend on transcription factor activation, gene expression and novel protein synthesis. Therefore, PAK plays a key role by phosphorylating Raf and Mek, two important kinases whose activities are dependent on Rho GTPases that phosphorylate CREB phosphorylation via Erk, the molecule downstream of Raf and Mek (Frost et al., 1997; King et al., 1998; Shin et al., 2002; Smith et al., 2008). Mek phosphorylation at Ser298 by PAK1 is essential for the synergistic effect of Rho GTPases on the Erk pathway and the increase in the upregulatory effect of Raf on Mek (Frost et al.,

1997; King et al., 1998). Moreover, Raf1 phosphorylation by PAK1, 2 or 3 at Ser338 is critical for Erk stimulation via Raf-1(Chaudhary et al., 2000; Zang et al., 2002).

A study using organotypic cultures of hippocampal pyramidal neurons has demonstrated that in PAK3 knockout cultures or in cultures treated with an antisense oligonucleotide to PAK3, abnormal dendritic spine and filopodia formation and damage to mushroom-type spine maturation were detected. These effects reproduce described cases of the human mental retardation phenotype (Purpura, 1974). Such abnormalities are also associated with reduced spontaneous activity and altered α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamatergic receptors, as well as LTP deficits. Altogether, this study demonstrated that transient suppression of PAK3 expression in pyramidal neurons resulted in an increase in the proportion of immature and non-functional dendritic spines, a defect in the establishment of synaptic contact, and stabilization of PSDs (Boda et al., 2006).

7. Participation of PAK in neuroplasticity

Synaptic plasticity is the cellular basis for learning and memory and involves mechanisms such as synaptic circuit remodeling, via either novel synapse formation or old synapse elimination to selectively strengthen or weaken the existing synaptic contacts (Yuste and Bonhoeffer, 2001; Sudhof and Malenka, 2008).

These processes involve adaptive changes in presynaptic active zones that control vesicle fusion and enable neurotransmitter release. The postsynaptic region also undergoes alterations, such as increases or decreases in the number of dendritic spines and modulation of the number of cell surface receptors, among others (Stevens, 2004; Newpher and Ehlers, 2009; Kasai et al., 2010; Kneussel and Wagner, 2013).

The processes of synaptic plasticity depend, among other factors, on the actin cytoskeletal dynamics, including the abundance of actin near the synaptic region. Actin filaments in dendritic spines are highly dynamic; they undergo continuous polymerization and depolymerization, constantly reorganizing themselves due to the consolidation of more stable networks and plasticity processes, such as long-term potentiation (LTP) (Hotulainen and Hoogenraad, 2010; Racz and Weinberg, 2012; Kneussel and Wagner, 2013).

The actin cytoskeleton also supports the trafficking of cell surface receptors in the postsynaptic cell and is associated with the recycling of synaptic vesicles in the presynaptic region (Hotulainen and Hoogenraad, 2010; Racz and Weinberg, 2012; Kneussel and Wagner, 2013). These mechanisms, which rely on the interactions between various cellular structural components, such as FLNa, myosin and actin, to provide structural stability, motor function and an efficient intracellular transport system for the cells which compose the synapse (Hotulainen and Hoogenraad, 2010; Korobova and Svitkina, 2010; Racz and Weinberg, 2012; Kneussel and Wagner, 2013).

PAK participates in the processes of stabilization and remodeling of cytoskeletal structures, such as lamellipodia, filopodia and stress fibers. These processes are indirectly upregulated by PAK via its activity on other molecules that participate in these processes, such as Mek, Raf and LIMK, leading to protein synthesis and actin polymerization, as well as directly regulated by PAK via its activity on structural components of the cytoskeleton, such as MLC and FLNa, as described above (Vadlamudi et al., 2002; Boda et al., 2008; Smith et al., 2008).

A mutation in PAK3 causes nonsyndromic intellectual disability (ID), or mental retardation (RM), because the inactivity of this kinase leads to changes in the morphology and function of dendritic spines. Furthermore, PAK3 gene silencing in hippocampal organotypic slice cultures resulted in a reduction in the number of mature dendritic spines, which provides strong evidence that the deficit in PAK3 activity caused a reduction in LTP (Fiala et al., 2002; Boda et al., 2004). Moreover, in another study by Boda et al. (2008), it was found that constitutively active PAK1 reversed the damage to spines caused by the inhibition of PAKs activity (Boda et al., 2008).

This abnormal phenotype of dendritic spines is observed in the neocortex of mice model RM. An increase in the proportion of immature and thin spines has been detected, as well as a reduction in the proportion of mature spines in the hippocampal dentate gyrus of these animals (Grossman et al., 2006, 2010; von Bohlen and Halbach, 2009).

A more recent study demonstrated a reduction in PAK activity in dendritic spines of mental retardation model mice. These animals were stimulated with hippocampal theta burst, and the activity of PAK and Rac1 was analyzed. Both proteins displayed decreased activity compared to control animals. A possible explanation is that these proteins are involved in the stabilization of newly formed actin filaments (Chen et al., 2010). Meng et al. (2005) did not detect phenotypic alterations in dendritic spines from brain tissue of PAK3 KO animals but in the same tissues, they detected a significant reduction in the late phase of LTP, an electrophysiological process importantly associated with learning and memory (Meng et al., 2005).

According to a study performed by Kreis (2007) the R67C, R419X and A365E mutations determine changes in different functional PAK3 regions and underlie mental retardation. The R67 mutation impairs PAK3 activation by Cdc42 and affects its subsequent activation by this GTPase. The R419X and A365E mutations completely abrogate PAK3 kinase activity. Both the R419X and A365E mutants slightly decrease the number of spines, but profoundly alters spine morphology, whereas expression of the R67C mutant dramatically decreases the spine density in rat hippocampal slices. These results highlight the importance of the combined activity of Cdc42 and PAK3 in the mechanisms of dendritic spine formation and synaptic plasticity (Kreis et al., 2007; Kelly and Chernoff, 2011).

In a study performed by Rex et al. (2009) evidence of the importance of the interaction between group I PAKs and Rac in LTP consolidation was provided. This study demonstrated that inhibition of the Rac-PAK interaction increases the period in which LTP is sensitive to disruption by latrunculin A after theta pulse stimulation (TBS) (Rex et al., 2009). PAK is a known actin filament regulator (Bokoch, 2003), and mice deficient in PAK3 exhibit impaired late phase LTP (Meng et al., 2005). It was also found that F-actin polymerization is stabilized via Rac-PAK signaling over a period of 2–10 min post-TBS (Rex et al., 2009; Panja and Bramham, 2013).

The group I PAKs participate in the processes of synaptic plasticity and are important for the mechanisms of spinogenesis and postnatal brain development (Kreis and Barnier, 2009;. Huang et al., 2011). Dysfunction of the group I PAKs has been detected in the brain of patients exhibiting mental retardation, and genetically altered mice not expressing PAK3 exhibited deficits in neuronal signaling. PAK3 knockout animals exhibited significant deficits in neuronal plasticity processes, such as LTP, and reduced learning and memory. However, the mechanism by which PAK3 regulates synaptic transmission and plasticity remains largely unknown (Thévenot et al., 2011; Ma et al., 2012).

A significant change in the levels of PAK1 and PAK3 have also been detected in brain tissue samples from patients suffering from Alzheimer's disease as well as animal models of this disease (Engler et al., 2006). Both PAK and PAK1-3 are typically diffusely distributed throughout the cell bodies and dendrites, but their expression levels are reduced in the cytoplasm of tissue from AD model animals. This result suggests that signaling disruption of

both PAKs may play an important role in dendritic spine deficits, synapse dysfunction and cognitive abnormalities in AD (Engler et al., 2006; Zhang et al., 2013).

Similar to that found for MR, in AD, spine morphology defects are early events that exert a negative effect on relevant circuit memory function, causing learning and cognitive deficits. In the brain of individuals suffering from AD, significant loss of PAK1 (35 ± 6%) and PAK3 (55–69%) were detected in the hippocampus, and the expression PAK3 was also significantly decreased in AD temporal cortex (63–77%) (Engler et al., 2006). In some cases, there was an increase in the total levels of PAK1-3 during the early stages of Alzheimer's disease, but a reduction in both the total cytoplasmic and phosphorylated PAK1 expression levels was detected during the final, AD stage, very serious (Duyckaerts et al., 2008; Zhang et al., 2013).

In cultured hippocampal neurons treated with β-amyloid, abnormal activation and decreased levels of PAK protein were observed in the cytoplasm, which was accompanied by a rapid loss of F-actin and dendritic spines. Along with this morphological change is the finding that hippocampal AD tissue also displays cells containing increased active cofilin, whereas the level of phosphorylated PAK is progressively reduced. The explanation for this rapid reduction in F-actin and dendritic spines is described above (Jonsson et al., 2013; Zhang et al., 2013).

8. Conclusion

As described in this review, it is clear that group I PAKs, especially isoforms 1 and 3, are importantly involved in the morphological regulation and the molecular behavior of neurons, with a key role in the processes of neuroplasticity. PAKs participate in the mechanisms of cytoskeletal regulation by interacting with cellular structural components, such as MLC and FLNa, and its activity modulates the dynamics and stability of the cellular structure. PAKs also participate in molecular cascades, such as the pathways of LIMK and ERK, resulting in increased levels of actin polymerization and synthesis of new proteins. This effect of PAKs on the cytoskeleton of neurons highlights its importance for the normal function of neurons and the effectiveness of neural networks involved in learning and many other cognitive functions. Therefore, changes in the activity of these kinases are potentially associated with deficits in learning and memory and diseases such as mental retardation, Huntington's disease and Alzheimer's disease. Therefore, we conclude that a better understanding of the function of PAKs relative to both their upstream and downstream signaling partners, as well as the physiological conditions in which its activity is relevant, can significantly contribute to a better understanding of various diseases or neurological deficits and also provides new information that can contribute to the diagnosis and treatment of these disorders.

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